851

Steroidal Sapogenins*

VII. Survey of Plants for Steroidal Sapogenins and Other Constituents

By MONROE E. WALL, MERLE M. KRIDER, C. F. KREWSON, C. ROLAND EDDY, J. J. WILLAMAN, D. S. CORELL, and H. S. GENTRY†

The results of the chemical examination of the first 1,000 plant samples received in a survey of plants for steroidal sapogenins are reported. Data are given on 925 samples, representing 292 identified species, 335 unidentified lots, 104 genera, and 30 families. Quantitative data are given for the occurrence of 14 steroidal sapogenins. Qualitative results were obtained for flavonols, alkaloids, tannins, and unsaturated sterols.

CORTISONE was originally isolated from the adrenal cortex. At present commercial cortisone is synthesized from certain bile acids of cattle. Since the supply of these is limited by the number of cattle slaughtered, a cheaper and potentially unlimited plant source of a suitable raw material for the production of this and other therapeutic agents would obviously be of inestimable value. Since 1948, academic, industrial, and governmental research agencies have endeavored to find new and improved starting materials for cortisone.

In 1929, an African species of the genus *Strophanthus*, presumably *S. sarmentosus*, was reported as containing sarmentogenin (1). Since sarmentogenin has an 11-hydroxyl group, it is potentially a much better cortisone precursor than bile acids (2). The National Institutes of Health (NIH), in following up this potential plant source for cortisone, requested the cooperation of the Bureau of Plant Industry, Soils, and Agricultural Engineering in obtaining raw materials of this genus and, in July, 1949, transferred sufficient funds to its Division of Plant Exploration and Introduction (PEI) to support an expedition in West Africa to search for materials of *Strophanthus* species for chemical analysis and propagation. John T. Baldwin, Jr., of the College of William and Mary, was employed to do this exploratory work.

From July, 1949, to June, 1950, Dr. Baldwin made collections of *Strophanthus* and other plants, including *Dioscorea*, in Liberia, Belgian Congo, Gold Coast, Nigeria, British and French

---

* Received March 16, 1953, from the Eastern Regional Research Laboratory of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Philadelphia, Pa.

† Monroe E. Wall, Merle M. Krider, C. F. Krewson, C. Roland Eddy, and J. J. Willaman are with the Eastern Regional Research Laboratory, Philadelphia, Pa., while D. S. Correll and H. S. Gentry are with the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Md.

In order to expedite the examination of such a large number of plant materials, groups of laboratory workers were organized, each group handling certain phases of the whole procedure. We gratefully acknowledge the work of J. W. Garvin, Walter Rumph, R. A. Pierce, H. M. Neilson, G. H. Eppley, Theodore Perlstein, H. E. Kenney, Arthur Finchler, H. W. Jones, M. L. McClennan, Samuel Serota, R. F. Mininger, H. I. Sinnamon, A. E. Jones, C. S. Fenske, M. K. Scott, M. A. Morris, L. P. Witnauer, A. Turner, Jr., and I. R. Necho.

Cameroons, and French Guinea. In the meantime, additional collections were obtained from various sources. To date, about 75 samples of *Strophanthus* and related genera have been obtained for chemical analysis by NIH, and over 100 collections, including about 25 species, are under propagation. A cooperative agricultural research program on *Strophanthus* with the Federal Experiment Station, Mayaguez, P. R., is now under way to determine the feasibility of growing *Strophanthus* as an agricultural crop. The highest yielding *Strophanthus* material discovered thus far is included in this program.

From the pioneering researches of R. F. Marker and his associates (3) it was known that steroidal sapogenins are readily converted to pregnane compounds with the desirable hormonal side chain. At first only steroids in which the 11-oxygen could be introduced chemically were useful. These included hecogenin with a 12-carbonyl group and diosgenin with unsaturation between carbons 5 and 6 (4, 5). More recently it has been shown that progesterone can be converted microbiologically to 11-α-hydroxy progesterone (6). Hence all progesterone precursors are potentially valuable. Among the steroidal sapogenins, diosgenin is outstanding but sarsasapogenin, smilagenin, and tigogenin can all be used for progesterone synthesis. Sapogenins with hydroxyl groups in positions other than carbon 3 and with 12-carbonyl groups are less satisfactory for this purpose

Marker, *et al.* showed that these sapogenins occur most frequently in plant families in the order Liliales. The several families of greatest interest are Amaryllidaceae, which includes *Agave*, the source of hecogenin; Dioscoriaceae, including primarily the genus *Dioscorea*, the source of diosgenin; and Liliaceae, including (among many other genera) *Yucca*, the source of other sapogenins. It was thus fortunate that Marker's fundamental research served as a preliminary guide as to what to look for and where to look for it.

PROCUREMENT

Following conferences of representatives of the National Institutes of Health, the Bureau of Plant Industry, Soils, and Agricultural Engineering, and the Bureau of Agricultural and Industrial Chemistry (BAIC), (the two latter of the Agricultural Research Administration, U. S. Department of Agriculture), it was decided that the combined forces of the three agencies could make an important contribution to the problem of increasing the cortisone supply. With authorization and funds from Congress, in the fall of 1950, the three agencies initiated their program: PEI to procure raw plant materials for chemical analysis and to try to develop promising species as crops; BAIC to isolate and chemically study potential cortisone precursors in the plant materials procured; NIH to synthesize cortisone from suitable plant steroids isolated by BAIC. Also NIH was to continue research on *Strophanthus* a. originally begun in July, 1949.

In the plant procurement phase, one of us (H. S. Gentry), in September, 1950, started exploration in Mexico and the southwestern United States for *Agave* species, since this region is the center of concentration for this genus. He also collected *Dioscorea* species in central Mexico, as well as a great deal of miscellaneous material. He still continues these North American explorations.

Other collectors who worked with him in Mexico are E. C. Ogden, University of Maine, from September, 1950 to June, 1951, and C. L. Gilly, Michigan State College, from January, 1951 to August, 1951. In addition to this work, Oliver W. Norvell, Carnegie Institution of Washington, explored in Guatemala, Honduras, El Salvador, and Nicaragua. In the meantime, several collaborators obtained materials from various regions in the United States. The principal ones were Robert R. Cruse, who obtained desert plants in the Tucson, Ariz., area; R. K. Godfrey, who obtained yuccas and miscellaneous materials in North and South Carolina; W. H. Duncan, who collected materials in Georgia and Florida; Barton H. Warnock, who obtained valuable materials from the Trans-Pecos region in Texas.

Since South Africa has one of the greatest concentrations of genera and species in the family Amaryllidaceae, Walter H. Hodge, now with PEI, was sent to this region from January to June, 1951. He collected materials from Cape Province, Natal, the Transvaal, and Southern and Northern Rhodesia.

Because of their increasing importance, concentration on collecting *Agave* and *Dioscorea* continues in the hope that the highest-yielding species or strains may be obtained with the idea of getting them into agricultural production as soon as possible. In order to avoid overlooking some other part of the plant kingdom which might give rich yields of sapogenins, plant materials from diverse families outside the Liliales are being chemically tested. These plant samples are not only acquired from nature but are being taken from a rich reservoir of plant materials maintained at the various plant introduction gardens of PEI.

Besides the cooperative agricultural program on *Strophanthus* and, lately, *Dioscorea* with the Federal Experiment Station, Mayaguez, P. R., PEI has a cooperative arrangement with the Firestone Plantations in Liberia to plant, maintain, and test *Strophanthus* in their plantations. Also, an arrangement has been made with the Huntington Botanic Garden, San Marino, Calif., to accept and maintain as a germ plasm center the various species and strains of *Agave* which Dr. Gentry and others are obtaining in the wild. Additional work is being planned at the Experiment Station, Sacaton, Ariz., to learn something of the agricultural possibilities of certain agaves. Besides the above, various tests and preliminary evaluations on *Dioscorea* and other genera are being made at the several plant introduction gardens maintained by PEI.

PROCEDURE

It was first necessary to devise a screening procedure which could be used on large numbers of samples. This procedure had to be as simple as possible, yet yielding correct identification of the sapogenins and, to a certain degree, quantitative information. The method adopted is given below. It was realized early that most of the plants to be submitted would be wild and that hence their chemistry would be either little known or unknown. In order to take advantage of this opportunity, a second procedure was established whereby the samples would be qualitatively examined for six other groups of constituents—flavonols, alkaloids, tannins, sterols, organic acids, and phenols.

The present report covers the first 1,000 accessions. For one reason or another some of these were discarded. Data are given on 925 samples, representing 292 identified and 335 unidentified species,[1] 104 genera, and 29 families. A search through Wehmer (7), Henry (8), Manske and Holmes (9), Chemical Abstracts, Biological Abstracts, and Marker's list (3) disclosed that there is no published chemical information on about half of the species listed. It is hoped, therefore, that the present compilation will prove to be a starting point for plant chemists looking for new constituents and new sources.

DETECTION AND ESTIMATION OF STEROIDS

Full details of the methods used for the detection and estimation of saponins and their derived sapogenins have been presented previously (10, 11). Hence this section will present only the broad outlines of the methods used and the interpretation of the results thus obtained.

Since complete and large-scale macro isolation (11) of steroidal sapogenins is time-consuming, and since many species are negative, all samples received were first put through the much more rapid micro screening procedure (10).

This has three distinct phases: (*a*) detection of saponins, (*b*) isolation of crude sapogenins, and (*c*) estimation of sapogenin content by infrared. A negative finding at any one of these three stages eliminated the sample and only samples positive in all three steps received the macro isolation.

Micro Procedure

Detection of Saponins.—This test and the other subsequent stages of the micro procedure could be carried out with as little as 5 Gm. dry or 20 Gm. wet sample. Where enough sample was available 50 Gm. dry or 200 Gm. wet were extracted with hot 95% alcohol, and made to 1 L.

Practically all saponins, whether steroidal or triterpenoid, have the property of hemolyzing red blood cells. If a 1-ml. aliquot of the above extract did not hemolyze 10 ml. of a saline red blood cell suspension, no significant quantity of steroidal sapogenin could be found by either micro or macro methods. Hence a negative hemolysis test automatically meant that the sample had no steroidal sapogenin but a positive test was not so clean cut.

[1] Subsequent papers in the series will list identifications as they are obtained.

Isolation of Crude Sapogenin.—An aliquot of the extract equivalent to 5.0 Gm. dry sample was concentrated and defatted with benzene. The saponins were hydrolyzed with 4 *N* hydrochloric acid at 75–80° for two hours and the resultant sapogenins extracted with benzene. After acetylation and alkali purification, the crude sapogenin acetate was dried and weighed. A yield of crude acetate less than 10–20 mg. meant less than 0.1% pure sapogenin, and the sample was classified as negative for our purposes and listed thus in this paper.

Infrared Estimation of Sapogenins.—Crude sapogenin acetates were dissolved in carbon disulfide or chloroform and their infrared spectra determined. The following information was thus obtained:

(*a*) Whether steroidal sapogenins were present in the sample from stage 2.

(*b*) If present, whether the content was low (less than 0.1%), moderate (0.1–0.5), or high (greater than 0.5).

(*c*) In favorable cases an indication of the kind and chemical configuration of the sapogenins present (usually when the content was greater than 0.5% and the sample relatively pure).

All samples showing less than 0.1% sapogenin by infrared were classified as negative for our purposes and are thus listed in this paper.

Macro Procedure

Positive samples from stage *c* above were carried through the macro procedure (10). This is essentially a larger-scale and more quantitative isolation, similar in principle to stage *b* above. The crude sapogenins were further purified with solvents, and mixtures were separated by chromatography. The content was determined from the total weight of purified sapogenin. The genins were identified by means of adsorption behavior, melting point, optical rotation, infrared and X-ray diffraction spectra, and if need be by characteristic chemical reactions. Occasionally a sample was found which contained no crystallizable material; such products were also classified as negative.

Hence a sample classified as 0% sapogenin in Table I may have received this rating in the four ways described above.

SCREENING TESTS FOR PLANT CONSTITUENTS OTHER THAN STEROIDS

Preparation of Extracts.—Alcoholic extracts remaining from the hemolytic tests were made ready for qualitative testing for other plant constituents as follows.

Aliquots, in sufficient amounts to give equivalents of 5–10 Gm. dry weight of original plant material, were evaporated to dryness. The residues were each dissolved in 25-ml. portions of hot water and filtered through serological filter pads. This procedure gave solutions representing about 300 mg. of plant per ml. Qualitative tests for alkaloids, tannins, organic acids, and phenols were made upon portions of these water extracts. Tests for sterols were made on chloroform extracts, prepared by dissolving residues from the alcoholic extracts in sufficient chloroform to give concentrations of plant material equivalent to the water extracts. Tests for flavonols were made upon the original alcoholic extracts of the plants.

An arbitrary system of scoring has been used to give a rough estimate of the amounts of the various constituents present, 0 indicating none found; one plus (+) a slight amount present; two plus (++), a moderate amount present; and three plus (+++), a substantial amount.

Flavonols.—The test applied is essentially that used by Willstätter (12) in his early studies on the reduction of quercetin. Beginning with the work of Stein (13) it was found that certain plant constituents gave distinctive color reactions when reduced in alcoholic solution with hydrochloric acid in the presence of metals such as sodium amalgam and magnesium. It has been established that this color reaction is due to the presence of the γ-benzopyrone nucleus. The details as used here have been set forth recently by Bryant (14). One milliliter of the alcoholic extract (representing 25–50 mg. of plant dry matter) was treated with 0.5 ml. hydrochloric acid (10%) and magnesium turnings. The color varied from pale to deep red depending upon the quantity of flavonoids. A score of one plus as used in this screening procedure indicated approximately 0.1% flavonoid present in the plant. This sensitivity was estimated by the addition of varying quantities of rutin to a known plant extract which initially gave a negative response to the test.

Alkaloids.—One milliliter of the water extract, prepared as described above, was acidified with 0.5 ml. of 1% hydrochloric acid and divided into two portions. To one was added 2 drops of Mayer's reagent (15, 16) [a potassium mercuric iodide solution described in (17)], and to the other was added 2 drops of silicotungstic acid (18)—12.0 Gm. of $4H_2O \cdot SiO_2 \cdot 12WO_3 \cdot 22H_2O$ in water sufficient to make 100 ml. If a precipitate was obtained either upon acidification or after addition of the reagents, a confirmatory test was performed in order to rule out interfering substances such as proteins.

About 1 ml. of water extract was made alkaline with 1% sodium hydroxide solution and extracted with an equal volume of chloroform. The chloroform extract was then extracted with an equal quantity of 1% hydrochloric acid and the latter extract divided into equal portions; one tested with Mayer's reagent, the other with silicotungstic acid solution. Scoring depended upon the quantity of precipitate obtained. On the basis of the silicotungstic acid test (using nicotine) a score of one plus indicated the presence of at least 0.01 to 0.1%, two plus indicated about 0.1 to 0.3%, and three plus over 0.3% alkaloids in the plant dry matter.

Tannins.—These were estimated by the response of the water extracts (prepared as described above) to gelatin-salt reagent (19) and confirmed by the black color produced with ferric chloride test solution (20, p. 939). Since precipitation with basic lead acetate occurred in every plant extract examined, whether or not tannin was found by other tests, this reagent was abandoned.

In the use of gelatin reagents a test was made with salt alone to guard against misinterpretation of results due to salting out effect which might be produced by nontannin constituents. Also, a separate test was made with 1% gelatin to aid in the evaluation of tannins since gelatin is less sensitive to them than gelatin-salt mixture and a positive test with gelatin alone indicates a larger amount of tannin present.

TABLE I.—OCCURRENCE OF THE VARIOUS SAPOGENINS IN THE SPECIES LISTED

Species	No. of Samples	Genin Content, M. F. B., % Min.	Max.
Chlorogenin			
Agave lecheguilla	1	...	0.1
Agave schottii	1	...	0.4
Agave sp.	2	tr	0.1
Chlorogalum pomeridianum	2	...	0.3
Yucca sp.	2	0.1	0.2
9-Dehydrohecogenin			
Agave deserti	1	...	tr
Agave huachucensis	1	...	0.1
Agave scabra	2	...	tr
Agave shawii	1	...	0.1
Agave sp.	1	...	0.2
9-Dehydromanogenin			
Agave goldmaniana	1	...	0.1
Agave nelsonii	1	...	tr
Agave shawii	2	tr	0.1
Diosgenin			
Agave sp.	1	...	0.2
Dioscorea composita	6	1.2	3.0
Dioscorea macrostachya	4	0.1	1.5
Dioscorea quaternata	2	0.3	0.4
Dioscorea sp.	7	0.1	2.0
Yucca filamentosa	1	...	0.3
Yucca sp.	1	...	0.1
Gitogenin			
Agave aurea	1	...	0.1
Agave filifera	1	...	0.2
Agave goldmaniana	1	...	tr
Agave nelsonii	1	...	0.1
Agave "pelon"	1	...	0.1
Agave schottii	1	...	0.8
Agave serrulata	1	...	0.1
Agave toumeyana	2	0.1	0.2
Agave sp.	8	0.1	0.3
Albuca sp.	2	tr	0.4
Chlorogalum pomeridiana	1	...	0.1
Yucca aloifolia	1	...	0.1
Yucca filamentosa	4	0.3	0.4
Yucca flacida	1	...	0.1
Yucca gloriosa	12	tr	0.2
Yucca thompsoniana	1	...	0.3
Yucca sp.	2	tr	0.1
Hecogenin			
Agave aurea	1	...	0.8
Agave chrysantha	1	...	0.5
Agave deserti	2	tr	0.1
Agave expansa	2	0.1	0.3
Agave goldmaniana	3	tr	0.2
Agave huachucensis	1	...	0.1
Agave nelsonii	2	...	0.1
Agave palmeri	1	...	0.5
Agave parryi	2	0.1	0.2
Agave cf. patonii	1	...	0.4
Agave scabra	2	0.1	0.2
Agave sebastiana	1	...	0.1
Agave serrulata	1	...	0.4
Agave shawii	2	0.1	0.2
Agave sobria	1	...	0.1
Agave toumeyana	4	0.1	0.3
Agave vexam	1	...	0.4
Agave sp.	14	tr	0.4
Yucca elata	1	...	0.1
Yucca gloriosa	3	tr	0.1
Yucca sp.	1	...	0.1

Species	No. of Samples	Genin Content, M. F. B., % Min.	Max.
Kammogenin			
Yucca filamentosa	3	...	0.1
Yucca gloriosa	1	...	0.1
Manogenin			
Agave aurea	1	...	0.2
Agave deserti	1	...	tr
Agave goldmaniana	4	tr	0.1
Agave mayoensis	1	...	tr
Agave nelsonii	1	...	tr
Agave cf. patonii	1	...	0.2
Agave serrulata	1	...	0.7
Agave shawii	2	tr	0.2
Agave toumeyana	2	0.1	0.2
Agave vexam	1	...	0.2
Agave sp.	1	...	0.2
Yucca filamentosa	1	...	0.1
Yucca gloriosa	5	tr	0.1
Sarsasapogenin			
Agave aff. attenuata	1	...	1.0
Yucca angustissima	1	...	0.3
Yucca baccata	3	0.2	0.5
Yucca elata	6	tr	0.7
Yucca gloriosa	1	...	0.2
Yucca intermedia	1	...	0.1
Yucca schidigera	2	0.4	0.5
Yucca standleyi	1	...	0.4
Yucca cf. thornberi	2	0.4	1.5
Yucca torreyi	1	...	0.5
Yucca valida	1	...	0.3
Yucca sp.	6	0.2	1.0
Yucca (leaf powder)	1	...	0.4
Samogenin			
Yucca carnerosana	1	...	0.3
Smilagenin			
Agave lecheguilla	5	0.1	1.0
Agave mayoensis	1	...	0.6
Agave aff. vilmoriniana	1	...	1.2
Agave sp.	8	0.2	0.9
Yucca aloifolia	1	...	0.1
Yucca carnerosana	1	...	0.1
Yucca sp.	1	...	0.1
Tigogenin			
Agave aurea	1	...	0.2
Agave goldmaniana	2	tr	0.1
Agave murpheyi	1	...	tr
Agave schottii	1	...	0.1
Agave serrulata	1	...	0.4
Agave shawii	1	...	tr
Agave sp.	6	tr	0.4
Albuca sp.	2	0.4	0.7
Chlorogalum pomeridiana	1	...	0.4
Yucca aloifolia	4	tr	0.2
Yucca elata	1	...	0.3
Yucca flacida	1	...	0.2
Yucca gloriosa	5	tr	0.2
Yucca sp.	4	tr	1.0
Yammogenin			
Dioscorea macrostachya	2	...	tr
Yuccagenin			
Agapanthus africanus	1	...	tr
Agapanthus umbellatus	2	0.1	0.2
Agave sp.	2	0.1	0.3
Nolina greenii	1	...	0.1
Yucca filamentosa	5	0.1	0.3
Yucca gloriosa	1	...	0.1
Yucca sp.	..	...	0.4

Since this Laboratory is interested in possible sources of tannins, C. W. Beebe made quantitative analyses (21) of some of the materials. Results obtained on several extracts are as follows:

Accession No.	Tannin, %	Screen Test Score
312	5.13	+
357	2.39	+
367	14.49	++
404	16.08	++
596	15.40	+++
727	18.04	+++
861	3.85	++
928	9.88[a]	++

[a] Analysis only approximate. Sample impossible to extract thoroughly. The scoring used in the screening procedure is in satisfactory agreement with the analytical values obtained.

Unsaturated Sterols.—Of the various color reactions for sterols (22) when they are treated with strong acids under dehydrating conditions, the Liebermann-Burchard and Salkowski tests were selected for this screening (23). Chloroform extracts prepared as described above were used and the scoring was made upon the basis of color intensities obtained. A score of one plus (+) indicated about 0.2% sterol content in the plant. This evaluation is based on the use of varying amounts of stigmasterol added to a plant extract which failed to produce the Liebermann-Burchard reaction. It should be pointed out, however, that the Liebermann-Burchard reaction is positive only in the presence of unsaturated sterols (24). Also, since the extracts tested had received no preliminary purification there is the possibility that other nonsteroidal unsaturated compounds (such as carotene and xanthophylls) may have interfered with this reaction (25). Where interference occurred, the color density appeared to be attained immediately. With spinasterol and similar sterols it did appear in this fashion. However, with most sterols and where interfering substances were not present, the color density was minimal immediately after the reaction began, then slowly increased, approaching a maximum in about fifteen to twenty minutes. In practically all of the samples tested in this screening work the color density was slow to develop and slow to reach a maximum.

Organic Acids and Phenols.—The term "organic acids" is used collectively in this work to apply only to water-soluble constituents of acid character. Five-milliliter portions of water extracts prepared as described under *Preparation of Extracts* were acidified with 2 ml. of 1% hydrochloric acid and extracted with 12 ml. of ether, the ether evaporated off spontaneously from a tared container, and the weight of air-dried residue recorded. The product has been termed "organic acids," but of course it would also contain nonvolatile phenols. Scoring of one-plus indicates less than 1% residue, two, 1–10%, and three, greater than 10%.

The qualitative scoring of phenols has been speculative. It has arbitrarily depended upon the tint and relative intensity of color produced with ferric chloride test reagent (20, p. 938). This test is of little value in the presence of tannins which produce a black color with ferric chloride. Absence

of organic acids above is indicative of the absence of nonvolatile phenols. The estimated value for phenols has therefore not been assigned higher than the figure given to organic acids.

RESULTS

A table has been prepared giving the data for each sample—its origin and identification, the results of the hemolysis tests, the kinds and amounts of steroidal sapogenins found, and the qualitative findings for flavonols, alkaloids, tannins, sterols, organic acids, and phenols. Because of space limitations this table cannot be given here. It has, however, been prepared in processed form as AIC–363 and may be obtained on request from the U. S. Department of Agriculture, Eastern Regional Research Laboratory, Bureau of Agricultural & Industrial Chemistry, Philadelphia 18, Pa.

Table I as included here is a rearrangement of the steroid data to show their occurrence by species.

A positive hemolysis test for saponin occurred in 60% of all species, but only one-fourth of these species contained steroidal saponins. There was a high incidence of steroidal sapogenins in certain genera: 47% in *Yucca*, 24% in *Agave* but, contrary to expectations, only 10% in *Dioscorea*. These steroids were found only in families of the Liliales—in the present list, Liliaceae, Amaryllidaceae, and Dioscoreaceae; two genera of Iridaceae had none. Of the 14 sapogenins found, diosgenin, gitogenin, hecogenin, sarsasapogenin, smilagenin, and tigogenin were in the greatest number of species. Kammogenin, samogenin, and yammogenin were rare and in small concentrations. Hecogenin was the predominant steroid in half the agaves; diosgenin in all of the yams; sarsasapogenin in 70% of the yuccas (26). *Y. filamentosa* was extremely varied, three different genins predominating in different samples.

The configuration at the ring A/B juncture at carbon 5 is with few exceptions a species characteristic. For example, many given *Agave* species form tigogenin, hecogenin, gitogenin, manogenin, and chlorogenin, singly or in a variety of combinations, all of which have the *trans* configuration at carbon 5. The corresponding *cis* form is never found in these species. *Agave lecheguilla* and a few other *Agave* species produce smilagenin with a *cis* structure at carbon 5, and the corresponding *trans* types are never found.

A similar situation is found in *Yucca*. Although a variety of sapogenins are produced by the various species, those which make the *cis* compounds never produce the *trans* and vice versa. In the *Dioscorea* the A/B juncture is a generic characteristic; only sapogenins unsaturated between carbons 5 and 6 are formed.

Regional specificity is particularly marked in *Yucca*. In agreement with Marker, *et al.* (3), we find that southwesterly yuccas growing in arid areas produce only sarsasapogenin. Those growing in the moist southeastern areas make a variety of sapogenins. In contrast to Marker, *et al.*, we find that smilagenin was not the predominant genin formed in the southeast. In most cases the sapogenins were C-5 *trans* compounds, tigogenin, and gitogenin being particularly common.

Flavonols were rarely found. A double-plus reaction was obtained only in four species of Liliaceae. A triple-plus was not obtained, although a sample of buckwheat leaves, *Fagopyrum esculentum* of about 4% rutin content, gave a triple-plus.

No alkaloids were found in *Agave*, but a triple-plus test was obtained in 14 other species of Amaryllidaceae, most of them from South Africa. A triple-plus was obtained in one species of Apocynaceae, in three of Dioscoreaceae, in two of Liliaceae, but not at all in *Yucca*. There is evidence that alkaloids do not occur in *Dioscoreas* native to the Western Hemisphere, but only in some Old World species (27). Table II lists the species in which alkaloids have been found for the first time.

TABLE II.—NEW ALKALOID-CONTAINING SPECIES

Amaryllidaceae
Ammocharis coranica (bulbs)
Ammocharis falcata (bulbs)
Brunsvigia rosea (whole)
Crinum buphanoides (bulbs)
Crinum cf. moorei (bulbs)
Haemanthus albiflos (bulbs)
Haemanthus coccineus (bulbs)
Haemanthus nelsonii (bulbs)
Leucojum aestivum (whole)
Narcissus cf. lazatta (bulbs, leaves)
Nerine angustifolia (bulbs)
Periphanes zeyheri (bulbs)
Vallota speciosa (bulbs)
Apocynaceae
Amsonia ciliata (whole)
Dioscoreaceae
Dioscorea dregeana (rhizome)
Liliaceae
Brodiaea uniflora (whole)
Polygonaceae
Rumex crispus (rhizomes)
Rumex hymenosepalus (rhizomes)

Tannin was absent from most families. In the Iridaceae it was found in four species of *Watsonia*. In the Liliaceae it was found in *Albuca*, *Nolina*, and *Smilax* (eight species). It was abundant in roots of *Rumex* (Polygonaceae), which is being considered as a commercial source.

Unsaturated sterols were generally frequent and abundant, mostly in leaves, but sometimes occurred in *Dioscorea* tubers.

A slight test for organic acids was obtained in practically all samples, as was to be expected. A two-plus test was obtained in only 32 samples, and these were mostly *Agave* and *Yucca*. One triple-plus was obtained in *Y. gloriosa*.

Even a slight test for phenols was obtained in only about 10% of the samples, and just one two-plus test, in *Y. gloriosa*.

Since these results for acids and for phenols seemed to lack significance or were fruitless, the tests for them were abandoned after the first 1,000 samples.

REFERENCES

(1) Jacobs, W. A., and Heidelberger, M., *J. Biol. Chem.* 81, 765(1929).
(2) Katz, A., *Helv. Chim. Acta*, 31, 993(1948).

(3) Marker, R. E., Wagner, R. B., Ulshafer, P. R., Wittbecker, E. L., Goldsmith, D. P. J., and Ruof, C. H., *J. Am. Chem. Soc.*, **69**, 2167(1947).
(4) Djerassi, C., Ringold, H. J., and Rosenkranz, G., *ibid.*, **73**, 5513(1951).
(5) Chamberlin, E. M., Ruyle, W. V., Erickson, A. E., Chemerda, J. M., Aliminosa, L. M., Erickson, R. L., Sita, G. E., and Tishler, M., *ibid.*, **73**, 2396(1951).
(6) Peterson, D. H., and Murray, H. C., *ibid.*, **74**, 1871 (1952).
(7) Wehmer, C., "Die Pflanzenstoffe," G. Fischer Verlag, Jena, 1929 and 1935.
(8) Henry, T. A., "Plant Alkaloids," 4th ed., The Blakiston Co., Inc., New York, 1949.
(9) Manske, R. H. F., and Holmes, H. L., "The Alkaloids. I.," Academic Press, Inc., New York, 1950.
(10) Wall, M. E., Krider, M. M., Roth, E. R., and Eddy, C. R., *J. Biol. Chem.*, **198**, 533(1952).
(11) Wall, M. E., Eddy, C. R., McClennan, M. L., and Klumpp, M. E., *Anal. Chem.*, **24**, 1337(1952).
(12) Willstätter, R., *Ber.*, **47**, 2831(1914).
(13) Stein, W., *J. prakt. Chem.* **85** (1), 351(1862); **88**, 80(1863); **89**, 491(1863).
(14) Bryant, E. F., THIS JOURNAL, **39**, 480(1950).
(15) Mayer, F. F., *Chem. News*, **7**, 159(1863).
(16) Mayer, F. F., *Am. J. Pharm.*, **25**, 20(1863).
(17) "Allen's Commercial Organic Analysis," Vol. VII, 5th ed., The Blakiston Co., Inc., New York, 1929, pp. 29–34.
(18) Bertrand, G., *Bull. soc. chim.*, **21** (3), 434(1899).
(19) Wilson, J. A., and Merrill, H. B., "Analysis of Leather and Materials Used in Making It," 1st ed., The McGraw-Hill Book Co., Inc., New York, 1931, pp. 290, 293.
(20) The United States Pharmacopeia, 14th Revision, Mack Publishing Co., Easton, Pa., 1950.
(21) "Methods of Sampling and Analysis of the American Leather Chemists Association. Vegetable Materials Containing Tannin. Proposed Methods," 1946, pp. A-1–A-14.
(22) Gortner, R. A., "Outlines of Biochemistry," 2nd ed., John Wiley & Sons, Inc., New York, 1938, p. 799.
(23) "Allen's Commercial Organic Analysis," Vol. II, 5th ed., The Blakiston Co., Inc., New York, 1924, p. 768.
(24) Fieser, L. F., "Chemistry of Natural Products Related to Phenanthrene," 2nd ed., Reinhold Publishing Corp., New York, 1937.
(25) Wall, M. E., and Kelley, E. G., *Anal. Chem.*, **19**, 677 (1947).
(26) *Anon., Chem. & Eng. News*, **30**, 2822 (1952).
(27) Willaman, J. J., Fenske, C. S., and Correll, D. S., *Science*, **118**, 329(1953).